

Salinity effects on protein content, lipid peroxidation, pigments, and proline in *Paulownia imperialis* (Siebold & Zuccarini) and *Paulownia fortunei* (Seemann & Hemsley) grown *in vitro*

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Abstract We evaluated the effects of saline stress on soluble proteins, lipid peroxidation (TBAR), chlorophyll *a*, chlorophyll *b*, β -carotene, violaxanthin, and proline in *Paulownia imperialis* and *Paulownia fortunei* plants grown *in vitro*. When the propagated plants reached a determined size, they were transferred aseptically to WPM culture medium containing different sodium chloride concentrations (0, 20, 40, 60, 80, and 160 mM) and were sampled at 15 and 30 days. Proline content was determined at 30 days after transfer only. Protein concentration significantly decreased with the highest salt levels in *P. imperialis* compared to controls in which no sodium chloride was added. In both *P. imperialis* and *P. fortunei*, lipid peroxidation significantly increased at 15 days but decreased at 30 days. Chlorophyll *a*, chlorophyll *b*, β -carotene, and violaxanthin significantly decreased with exposure to higher sodium chloride concentrations at 15 and 30 days in both species. Proline content in *P. imperialis* significantly increased in plants grown in 20 and 40 mM of sodium chloride and decreased in higher sodium chloride concentrations. In *P. fortunei*, this measure significantly decreased proline content at all salt concentrations in plants exposed to all levels of sodium chloride compared to controls. Our results show that *P. imperialis* is more tolerant to salt stress at the salinity conditions tested.

Keywords: *Paulownia fortunei*, *Paulownia imperialis*, pigments, proline, proteins, salinity, TBARS, tissue culture

INTRODUCTION

One third of the world's agricultural land is damaged, and approximately 5% of 1.5 Bha of cultivated land is affected by salt (Tabatabaei, 2006). Environmental degradation, mainly soil pollution and erosion, desertification, declining biodiversity, and overuse of agrochemicals cause biotic and abiotic stress in plants (Shao and Chu, 2005); among those stresses that limit plant growth and development, drought is a major factor and is closely related to salt stress (Song et al. 2008). Abiotic stress,

particularly salinity and drought are major stresses that cause crop losses worldwide (Vinocur and Altman, 2005). Plant responses to salt and water stress involve many macromolecules and small compounds, such as proline (Shao et al. 2006). Salinity in agricultural land limits crop productivity due to irrigation practices with bad quality water (Chinnusamy et al. 2005); sodium chloride is the most abundant salt that contributes to soil salinity (Koca et al. 2007). High salinity causes severe damage to plants, including growth inhibition, impaired metabolism, necrosis, and loss of production and quality (Sivritepe and Eris, 1999). In addition to the toxicity caused by ions, salinity and osmotic stress cause an imbalance of nutrients in plants (Cavagnaro et al. 2006; Song et al. 2006). The imbalance caused by salinity affects the nutrients involved in protein synthesis and those involved in photosynthesis, which can lead to inhibition of these processes (Vieira-Santos et al. 2001), as well as to the degradation of pigments chlorophyll *a* and *b* (Di Martino et al. 2003).

Research to improve salt tolerance in plants is mainly focused on biochemical and physiological aspects. The genes responsible for salt tolerance in some species have been identified (Schleiff, 2008), but stress might occur as a complex mechanism of several interacting environmental factors that cause variations in plant phenotypes, as plants respond to complex growth conditions (Shao et al. 2007a). Salt stress causes the plants to suffer dehydration, which is one of the abiotic stresses in plants.

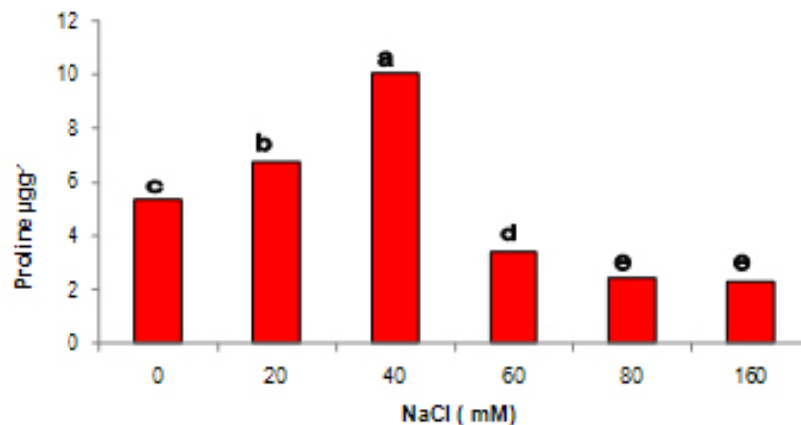


Fig.1 Effect of sodium chloride (mM) on proline (µg/g) in *P. imperialis* at 30 days. Values not associated with the same letter are significantly different ($P < 0.05$).

It is known that osmotic stress induces oxidative damage, reducing biosynthesis of compound osmoprotectors; basically plants record their environment and respond with resistance or adaptation mechanisms (Shao et al. 2007b; Shao et al. 2008). On the other hand, plant stress mechanisms are very different depending on the degree of stress tolerance, growth, and different development stages, evolution in time, materials and experimental plots, which increase their complexity (Shao et al. 2005a). To protect and preserve osmotic stability, cells accumulate proline that is probably the most widely osmolyte found in plants and other organisms (Bartels and Sunkar, 2005). Proline is a compound that tends to accumulate in response to metabolic salt stress (Cushman, 2001); it is thus important in the osmotic adjustment in plants under

stress (Di Martino et al. 2003; Mademba et al. 2003) and serves as an osmoprotectant (Benavides, 2002; Wanichananan et al. 2003). It is understood that proline is a consistent and compatible solute that does not inhibit enzymatic activities (Rathinasabapathi, 2000) at high concentrations, and facilitates biochemical reactions (Tester and Davenport, 2003). Cavagnaro et al. 2006 have suggested that culture *in vitro* is an appropriate system for studies on salinity in plants because it easily recapitulates the results found in the field and in greenhouses where environmental conditions are controlled. Plant cell and tissue culture methods could be useful in studying salinity tolerance mechanisms in plants and their effects on crop production when they are not evidently known (Akinci and Simsek, 2004).

Paulownia trees are native to China and have spread to other countries, such as Korea, Japan, Australia, and the United States. They can reach up to 30 meters at maturity (Bonner, 1995), have a significant economical value because they produce very high quality wood (Bergmann, 1998), and they can be used in paper pulp manufacture. These characteristics make the tree suitable for its use in furniture and poles. Even though the wood is light, it can be processed cost-effectively, as it does not contain resins or oils. In addition, it can be used as fodder and is economically important when used in intercropping systems (Jianbo, 2006). *Paulownia imperialis* (syn. *P. tomentosa*) has become very important in the United States after its naturalization over 150 years ago (Bergmann, 2003). *Paulownia fortunei* is a potential agroforestry species (Muthuri et al. 2005) that can reach a height of 8 m in five years; its timber is used for microclimatic improvement, forestation, and remediation.

The purpose of this investigation was to determine the sodium chloride tolerance of *P. imperialis* and *P. fortunei* based on analyses of soluble proteins, lipid peroxidation, chlorophyll *a*, chlorophyll *b*, β -carotene violaxanthin, and proline content in plants grown *in vitro*.

MATERIALS AND METHODS

Tissue culture

Culture WPM (Woody Plant Medium) (Trigiano and Gray, 2000) was prepared with 3% sucrose, and pH was adjusted to 5.8. Agar (0.8%) was added (Difco Bacto agar), medium was placed in 20 ml vessels, and the vessels and medium were sterilised at 121°C and 15 lb of pressure for 15 min.

Plant growth

The seeds of *Paulownia imperialis* and *Paulownia fortunei* (from Sandeman Seeds, France) were disinfected with 95% ethyl alcohol (v/v) for 5 sec and 20% NaOCl for 5 min. Seeds were then rinsed four times in sterile distilled water. The seeds were inoculated aseptically in a laminar flow chamber (Edge Gard Hood). The cultures were incubated in a growth chamber at 25 \pm 2°C at a relative humidity of 70% under 16 hrs of light.

After two months under these conditions, the plants were transferred aseptically to a new culture medium containing WPM with sodium chloride at concentrations of 0, 20,

40, 60, 80, and 160 mM. Ten vessels were prepared for each treatment, one plant per vessel and placed in the controlled incubation conditions mentioned above.

Protein determination

Protein determination was made according to the Bradford method (Bradford, 1976) and using a standard curve prepared with bovine serum albumin. Briefly, Coomassie blue, in response to protein concentration, reacts with basic amino acid residues, especially arginine. When the plants had been treated with salt conditions for 15 and 30 days, 100 mg of the dried aerial portion was placed in a test tube with 2 ml of 50 mM potassium phosphate buffer at pH 7.0. Plant tissues were centrifuged at 7000-12000 rpm. The supernatant was recovered and centrifuged at 3000 rpm for 15 min at 4°C. Samples were diluted 1:100 and read in triplicate in a spectrophotometer at 595 nm.

Lipid peroxidation (TBARS) determination

For each sodium chloride concentration, the aerial part of three plants was cut and dried at 15 and 30 days to quantify malondialdehyde (MDA) production. Lipid hydroperoxides and aldehydes resulting from peroxidation of the cell membrane react with thiobarbituric acid (TBA) to form MDA, which is a crystalline pink pigment with absorption from 525 to 535 nm (Persky et al. 2000). To assay MDA production, 2 ml of 0.9% saline solution at pH 7 was added to tubes. The tubes were kept on ice, and 100 mg of dried tissue was added. The tissues were centrifuged at a speed from 7000 to 12000 rpm. Then, 250 µl of each supernatant was added to 1.5 ml Eppendorf tubes. All samples were analysed in triplicate. Samples and standards were incubated at 37°C in a water bath for fifteen minutes. The tubes were then removed and placed in an ice bath to stop the reaction. Five hundred microlitres of TBA (thiobarbituric acid) solution were added to each tube (Sigma T5500). Samples were kept under constant stirring with a vortex. The tubes were placed in a shaking water bath at 90°C for ten min. The tubes were kept closed and placed in an ice bath until cool and then centrifuged at 3000 rpm for 10 min at 4°C. Each supernatant (1.5 ml) was transferred to a plastic cell for analysis in a spectrophotometer (Jenway 6505 UV / Vis) at 532 nm (Persky et al. 2000).

Table 1. Effect of sodium chloride (mM) on protein levels (mg/l) in *Paulownia imperialis* and *Paulownia fortunei* at 15 and 30 days. Letters denote a statistically significant difference ($P < 0.05$) in levels of proteins at 15 and 30 days. Values not associated with the same letter are significantly different ($P < 0.05$).

NaCl (mM)	<i>Paulownia imperialis</i>		<i>Paulownia fortunei</i>	
	Days		Days	
	15	30	15	30
0	1.075 ± 0b	0.109 ± 0.01b	0.350 ± 0b	0.109 ± 0.01c
20	1.439 ± 0a	0.182 ± 0 a	0.53 ± 0.26b	0.076 ± 0.01d
40	1.196 ± 0.2b	0.109 ± 0.01b	1.196 ± 0.21c	0.134 ± 0.01c
60	1.075 ± 0b	0.096 ± 0.02bc	0.712 ± 0b	0.114 ± 0.01c
80	1.075 ± 0b	0.076 ± 0.01c	2.16 ± 0a	0.153 ± 0 abc
160	0.591 ± 0.2c	0.172 ± 0.01a	1.80 ± 0.51a	0.172 ± 0.01a

Pigment determination

The pigments were analysed using HPLC (High Performance Liquid Chromatography). Samples were collected from the aerial parts of three plants grown in varying concentrations of sodium chloride at 15 and 30 days of incubation. Samples of the control plants for both *Paulownia* species were also collected at these times. For pigment extraction, 1-3 mg of freeze-dried material from the aerial part of each plant was extracted with 1 ml acetone and weighed. The samples, covered with aluminium foil to protect them from light, were incubated for 24 hrs at -20°C. Thawed samples were centrifuged at 4000 rpm for 15 min at 5°C. The extract was filtered through a 0.45 µm fibreglass membrane. The filtrate was recovered in Eppendorf vials and stored at -20°C. Then 20 µl of each sample was injected into the high performance liquid chromatographer (HPLC Model 1100, Hewlett Packard, Palo Alto, C.A.).

For pigment separation, we used the method described by Vidussi et al. 1996, using a mobile phase and combining two solutions. The first solution (A) was a mixture of methanol and 1 N ammonium acetate at a 70:30 ratio (v/v), and the second solution (B) was 100% HPLC grade methanol. The stationary phase used was a Hypersil C8 column that was 10 cm in length, 0.45 cm in diameter, and filled with 5 µm particles of silica. The detector used was an array of diodes with a wavelength range of 190-900 nm and the capacity to determine five fixed wavelengths. Quantification was done by generating a standard curve with concentrations of 20, 40, 60, 80, and 100 ng/ml of each pigment. The pigments identified were chlorophyll *a*, chlorophyll *b*, β-carotene, and violaxanthin.

Proline determination

Proline content was analysed after the plants had grown for 30 days under the various salinity conditions. The proline analysis was conducted using HPLC (Varian, Palo Alto, CA. Model 5000), according to the technique described by Vázquez-Ortiz et al. (1995). Samples were prepared from the aerial parts of three plants for each sodium chloride treatment. Extracts for analyses were prepared by blending 1.0 g of the plant tissue with 2.0 ml of trichloroacetic acid (TCA) (Sigma T6399) in a blender (VWR). The extracts were centrifuged and the supernatants were mixed with 0.4 M of borate buffer solution and NBD-Cl (7-chloro-4-nitrobenzo 2 oxa-1,3-diazol-) (2 mg/ml in methanol). Extracts were mixed and maintained at 60°C for 5 min. Then the reaction was stopped by adding 50 µl of 1M HCl and cooled at 0°C for 30 min. Ten microlitres of each sample were injected in the HPLC; the column used was a Lichrosor RP-18, 5 µm (30 cm x 4.6 mm ID). The mobile phase (A) was prepared with 0.1 M sodium acetate buffer, pH 7.2, using methanol and tetrahydrofuran (900:95:5 v/v/v) (Sigma Chemical Co.) as organic modifiers. The mobile phase solvent (B) was methanol (Merck). Solvents were filtered through 0.22 µm filters (Millipore Corporation). Proline (Pierce Chemical Co.) was used to generate a standard curve. The flow was 1 ml/min from 25 to 29°C, and the retention time for proline was 5 min.

Statistical analysis

We used a completely randomised design with three replicates. To determine significant differences among treatments, we used the variance analysis of one of them. LSD was used to compare the mean values with a confidence level of 95%.

The results were statistically analysed using the StatGraphic Version 1.4 Plus program.

RESULTS AND DISCUSSION

Protein determination

Paulownia imperialis. Protein content was higher in plants grown in the lowest concentration of sodium chloride (20 mM) (Table 1) at 15 days. Protein content was statistically similar in plants grown at concentrations of 40, 60, and 80 mM of sodium chloride, and significantly decreased at the highest sodium chloride concentration (160 mM). At 30 days, the protein content of the control plants and that of the plants grown with 40 mM of sodium chloride did not show significant differences compared to that of the plants treated with 60 mM. Protein content significantly decreased in plants treated with 80 mM of sodium chloride and increased in plants grown in 160 mM. One characteristic of saline stress is the removal of potassium ions by plant roots, which causes a physiological imbalance because potassium is necessary for protein synthesis. Potassium loss causes diminished plant growth and development (Chen et al. 2007). If the stress is prolonged, it could affect protein synthesis and eventually cause it to decline (Caplan et al. 1990).

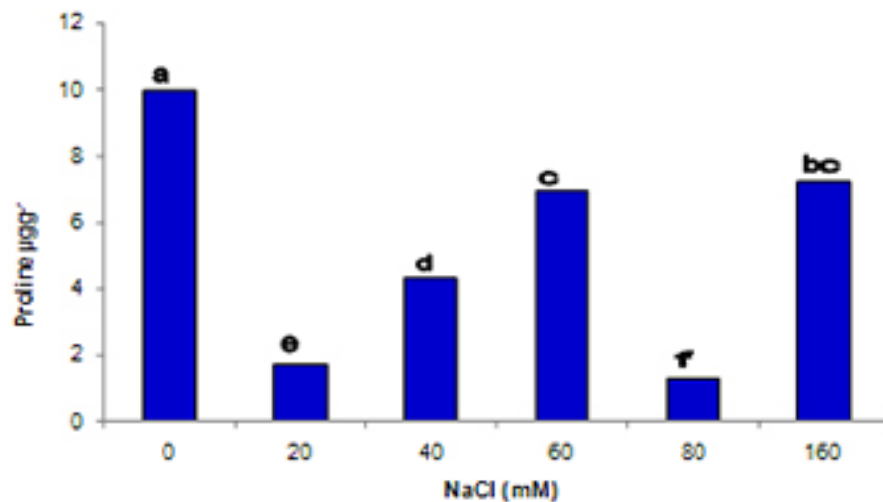


Fig. 2 Effect of sodium chloride (mM) on proline ($\mu\text{g/g}$) in *P. fortunei* at 30 days. Values not associated with the same letter are significantly different ($P < 0.05$).

Paulownia fortunei. Plants grown in higher salinity levels (80 and 160 mM of sodium chloride) showed significantly higher levels of soluble protein at 15 days (Table 1), while plants grown at lower sodium chloride levels showed statistically similar concentrations of soluble proteins compared with those of the control plants. At 30 days, the highest soluble protein concentration was observed in plants grown at higher sodium chloride levels (160 mM). Protein content may have increased because

plants have some tolerance level at these sodium chloride concentrations. The decrease in protein content is due to the effects of sodium chloride on protein synthesis (Omar et al. 1993). Furthermore, prolonged stress could affect protein synthesis and provoke its decline (Caplan et al. 1990). Protein content in *Vigna unguiculata* (L) Walp. Vita 5 was not affected compared to controls, while it significantly increased in the stems of plants grown with 100 mM of sodium chloride (Franco et al. 1999). To survive under stress, plants accumulate proteins that protect cells from stress effects (Wang et al. 2003); we observed higher protein concentrations in *P. imperialis* than in *P. fortunei*. These results show that *P. imperialis* is more tolerant to salinity.

Lipid peroxidation (TBARS)

TBAR content was unstable in *Paulownia imperialis* plants grown in increased levels of sodium chloride at 15 days. Control plants and plants that were grown in 40 mM of sodium chloride showed similar results (Table 2). Lipid peroxidation levels may indicate osmotic stress due to increased proline content (Figure 1). After 30 days, the control showed the highest value and lipid peroxidation decreased in plants grown in increasing sodium chloride concentrations. In two wheat cultivars (Sardari and Alvand), lipid peroxidation increased with 100, 150, and 200 mM of sodium chloride in Alvand; however, in the Sardari cultivar lipid peroxidation increased with 50 mM NaCl, decreased with 100 mM NaCl, and remained unchanged with increasing salt level (Esfandiari et al. 2007).

In *P. fortunei*, TBAR content significantly declined in plants grown with increasing levels of sodium chloride. At 20 mM of sodium chloride, the TBAR content did not vary with respect to the value obtained for the control plants at 15 days. At 30 days, TBAR content significantly increased in plants grown with 20 mM of sodium chloride, and it significantly declined in plants grown at the other salinity levels (Table 2). These

Table 2. Effect of sodium chloride on lipid peroxidation (nmolTBARSmg/protein) in *Paulownia imperialis* and *Paulownia fortunei* at 15 and 30 days. Letters denote a statistically significant difference ($P < 0.05$) in levels of lipids peroxidation at 15 and 30 days. Values not associated with the same letter are significantly different ($P < 0.05$).

NaCl (mM)	<i>Paulownia imperialis</i>		<i>Paulownia fortunei</i>	
	Days		Days	
	15	30	15	30
0	359.7 ± 34.1c	9407.58 ± 959.52a	5787.97 ± 530.67a	20270.08 ± 2380.7b
20	924.25 ± 69.49b	5676.98 ± 660.18b	4925.33 ± 2197.92a	36674.24 ± 7443.71a
40	581.61 ± 188.72bc	2830.83 ± 1383.68c	3497.01 ± 652.73ac	7970.78 ± 2735.98cd
60	3683.7 ± 604.22 a	3980.91 ± 2408.76bc	2805.7 ± 253b	11957.89 ± 2230.53c
80	1252.71 ± 51.23b	4209.09 ± 1429.57bc	1163.58 ± 78.88c	3688.68 ± 587.05d
160	3703.2 ± 260.62a	2340.64 ± 215.74c	1141.96 ± 24.47c	3855.37 ± 617.08d

results are not consistent with experiments performed with onions (*Allium cepa* L.), where TBAR content increased with salinity (El-Baky et al. 2003), or in experiments with cowpea plants (*Vigna unguiculata* (H) Walp), where an increase in TBAR content was noted in plants grown with 200 mM of sodium chloride (Cavalcanti et al. 2004). In *Carrizo citrange* plants, lipid peroxidation increased with 30, 60, and 90 mM NaCl (Arbona et al. 2003).

Pigment content quantitation

P. imperialis. Higher salinity significantly reduced chlorophyll *a* content as observed at both sampling times (15 and 30 days) (Table 3). At 15 days, chlorophyll *b* content in plants grown at 20 and 40 mM of sodium chloride did not vary with respect to controls. High sodium chloride concentrations resulted in a significant decrease in chlorophyll *b* (Table 3). At 30 days, chlorophyll *b* significantly decreased with all the sodium chloride concentrations tested. Decreases in both pigments were reported by Di Martino et al. (2003) in spinach (*Spinacia oleraceae*) exposed to salinity stress.

β -carotene content significantly decreased in plants grown in high concentrations of Sodium chloride at day 15 (Table 3). However, after 30 days of growth in the lowest level of sodium chloride (20 mM), β -carotene content was equal to that in control plants. β -carotene levels significantly decreased in plants grown with increasing levels of salinity.

At 15 and 30 days, violaxanthin content (Table 3) significantly decreased in all plants grown in salinity conditions compared with the control plants. In work done with the halophyte *Atriplex centralasiatica* with sodium chloride concentrations ranging from 0 to 400 mM, the content of chlorophyll *a + b* and β -carotene remained unchanged at increased sodium chloride concentrations (Qiu et al. 2003).

P. fortunei. Table 4 shows that chlorophyll *a* content in control plants was similar to that in plants that were grown in 20, 40, and 60 mM of sodium chloride at 15 sampling days. It significantly decreased with 80 and 160 mM of sodium chloride at 15 days, while at 30 days, the pigment fell significantly in increased salinity.

Table 3. Effect of sodium chloride (mM) on pigments (ng/ μ g) in *Paulownia imperialis* at 15 and 30 days. Letters denote a statistically significant difference ($P < 0.05$) in levels of pigments at 15 and 30 days. Values not associated with the same letter are significantly different ($P < 0.05$).

NaCl (mM)	Chlorophyll <i>a</i>		Chlorophyll <i>b</i>		β -carotene		Violaxanthin	
	Days		Days		Days		Days	
	15	30	15	30	15	30	15	30
0	6.455a	3.18a	1.66a	0.909a	0.62ab	0.177a	0.398a	0.149a
20	4.766b	1.5bc	2.3a	0.466b	0.55ab	0.129ab	0.282b	0.063b
40	6.143ab	1.54bc	1.64a	0.479ab	0.74 a	0.078bc	0.372ab	0.065b
60	2.529c	0.57bc	0.75b	0.248b	0.25bc	0.050bc	0.143c	0.02b
80	2.27c	0.09c	0.77b	0.051b	0.24bc	0.007c	0.144c	0.003b
160	1.458c	0.68c	0.45b	0.224b	0.19c	0.027c	0.094c	0.032b

Chlorophyll *b* contents in plants exposed to 20 mM levels of sodium chloride were similar to those observed in controls. Chlorophyll *b* content fell significantly at higher sodium chloride concentrations after 15 days. Salt treatment significantly reduced chlorophyll *b* at 30 days (Table 4). Plants presented necrosis at 30 days, and thus chlorophyll content was affected. In strawberry plants exposed to 0, 8.55, 17.10, and 34.21 mM of sodium chloride, total chlorophyll content did not change (Turhan and Eris, 2005), whereas in sweet cherry rootstock Gisela 5 (*Prunus cerasus* x *Prunus canescens*) a decrease in chlorophyll content was observed as a result of sodium chloride exposure (0, 50, 100, and 150mM) (Erturk et al. 2007). Stoeva and Kaymakanova (2008) observed with beans that chlorophyll *a*, chlorophyll *b*, and carotenoids decreased with increasing salinity (100 mM NaCl).

At day 15, *P. fortunei* plants grown at 80 and 160 mM of sodium chloride had significantly diminished β -carotene content. After 30 days, this pigment significantly decreased in plants grown in sodium chloride at all concentrations tested (Table 4). At day 15 the violaxanthin content in the control was the same as that shown by plants that were grown in 20 and 40 mM of sodium chloride. It significantly decreased in plants grown with increasing sodium chloride concentrations (Table 4).

Proline determination

In *Paulownia imperialis*, proline content significantly increased in plants exposed to increased sodium chloride levels. Plants grown in 40 mM of sodium chloride showed the highest proline content. Proline accumulation in tissues of plants exposed to saline stress can be attributed to an increase in proline synthesis and a reduction in its degradation. With higher sodium chloride levels (60, 80, and 160 mM) (Figure 1), a significant decline in proline content was observed. This is consistent with the experiments of Lutts et al. (2002) in rice cultivars that are sensitive to salt stress. In these experiments, proline content in leaves increased in plants exposed to 50 mM of sodium chloride and decreased in plants exposed to 100 mM. Similar results were obtained in experiments made with *Sapindus trifoliatus* L. somatic embryo using different sodium chloride concentrations (Unnikrishnan et al. 1991). In our experiment proline content decrease could have been due to greater proline utilization than synthesis caused by NaCl stress.

In *P. fortunei*, proline content significantly decreased in plants grown at all sodium chloride concentrations, compared to controls. In particular, we observed less proline at the lowest salinity level (20 mM) (Figure 2). This result agrees with data presented by Mademba et al. (2003) who suggest that proline accumulation helps to stabilise proteins at high ionic strength or at low water activity. In experiments conducted with sugar beets (*Beta vulgaris* L.) grown at different sodium chloride concentrations, similar results have been reported (Gzik, 1996). In *Hordeum marinum* and *Hordeum vulgare*, exposure to increasing sodium chloride concentrations also caused an increase in proline content (Garthwaite et al. 2005). Al-Khayri (2002) observed that in experiments on date palm callus a significant proline accumulation occurred when increasing sodium chloride (0-225 mM). With *Carrizo citrange* proline increased with 30, 60, and 90 mM of NaCl (Arbona et al. 2003).

Dracup (1991) mentioned that proline levels increase with increasing salt concentrations. This is consistent with our observations in *P. imperialis* grown in

sodium chloride concentrations of 40 mM or less. In *P. fortunei*, proline accumulation significantly decreased in plants grown in all sodium chloride concentrations (Figure 2). It is significant that this species is less tolerant to salinity than *P. imperialis*. Leaves of *Suaeda physophora* and stems of *Haloxylon persicum* grown with 300 mM of sodium chloride did not show significant changes in proline content (Song et al. 2006). In this experiment, proline accumulation in plants grown with 40 mM of sodium chloride may be due to the development of a stress tolerance mechanism to sodium chloride in *P. imperialis*. However, with increasing salt concentrations, proline content declined (Figure 1). Experiments with different varieties of sorghum (*Sorghum bicolor*) showed that proline was not associated with tolerance to saline stress (Caplan et al. 1990), whereas with rice (*Oryza sativa* L.), proline contributed to growth inhibition and a decline of chlorophyll levels in response to salinity (Garcia et al. 1997). In experiments with beans (*Phaseolus vulgaris*, L.), there was an increase in proline content with increasing salinity (100 mM NaCl) (Stoeva and Kaymakanova, 2008). In experiments with *Citrus*, it has been noted that the excess of sodium and chloride ions could act as inhibitors of proline response, and their accumulation could contribute to osmotic adjustment (Mademba et al. 2003). Proline content decline in plants exposed to higher salinity could be due to a greater use of proline by the plants. Both species of *Paulownia* responded differently to sodium chloride stress regarding proline accumulation.

Our results show that with increasing length of exposure to the sodium chloride levels used, pigment contents declined in both species. Activity inhibition of soluble proteins, proline, and lipid peroxidation, as well as the decrease in chlorophyll and carotenoids contents, indicate damage caused by sodium chloride at concentrations of 60 mM and higher in *P. imperialis* and *P. fortunei*. The increased TBARS value shown by *P. imperialis* after 15 days may indicate lipid peroxidation of the cell membrane components caused by reactive oxygen species that are generated by oxidative stress. When oxidative stress is severe and prolonged, it can produce cell death, which was observed in this study in plants grown in higher levels of salinity with both species of *Paulownia*.

Table 4. Effect of sodium chloride (mM) on pigments (ng/μg) in the aerial parts of *Paulownia fortunei* at 15 and 30 days. Letters denote a statistically significant difference ($P < 0.05$) in levels of pigments at 15 and 30 days. Values not associated with the same letter are significantly different ($P < 0.05$).

NaCl (mM)	Chlorophyll a		Chlorophyll b		β-carotene		Violaxanthin	
	Days	Days	Days	Days	Days	Days	Days	Days
	15	30	15	30	15	30	15	30
0	6.455a	3.18a	1.66a	0.909a	0.62ab	0.177a	0.398a	0.149a
20	4.766b	1.5bc	2.3a	0.466b	0.55ab	0.129ab	0.282b	0.063b
40	6.143ab	1.54bc	1.64a	0.479ab	0.74 a	0.078bc	0.372ab	0.065b
60	2.529c	0.57bc	0.75b	0.248b	0.25bc	0.050bc	0.143c	0.02b
80	2.27c	0.09c	0.77b	0.051b	0.24bc	0.007c	0.144c	0.003b
160	1.458c	0.68c	0.45b	0.224b	0.19c	0.027c	0.094c	0.032b

P. imperialis is tolerant to sodium chloride at 40-60 mM of sodium chloride concentrations. Further investigations are needed to determine the influence of sodium chloride on *Paulownia* species, in order to elucidate the mechanisms utilised by these species. With wheat it was found that the development stage is important for stress response (Shao et al. 2005b). The osmotic adjustment is the main component of the physiological machinery to which plants respond to water stress; it has been reported that with high proline and low MDA levels, wheat cultivars resisted better in drought conditions (Shao et al. 2006).

Probably stress-tolerant plants accumulate compatible solutes that will be generated in other species. For practical application in crop plants, the synthesis capacity for a particular solute must be consistent and improved. Increases in expression levels on plant and availability of substrate in a specific subcellular compartment must facilitate the generation of plants that produce high levels of certain compatible solutes and are thus tolerant to multiple stresses (Chen and Murata, 2002). Plant response to salinity varies with climate and growing conditions, crop management and irrigation, variety, and sensitivity of the crops in different growth stages (Chartzoulakis, 2005).

During plant growth in desert areas, there are important conditions which limit the factors that influence plant growth and development and affect production and quality, as drought and salinity (Shao and Chu, 2005). In all life processes a mutual relationship between plant response and the environment is very important and is the essential basis for different types of sustainable development in the world, particularly for sustainable agricultural development.

In other studies (Ayala-Astorga et al. 2009) with both *Paulownia* species grown *in vitro*, it was reported they survived at a NaCl concentration of 60 mM, and that higher concentrations induced necroses and death. These results indicate that both species are slightly tolerant to sodium salinity. It has also been reported that *P. imperialis* was more resistant than *P. fortunei* in studies of physiological variables under drought conditions with both species of *Paulownia* growing at pots and in the field (Llano-Sotelo et al. 2010).

Zhang et al. (2009) suggested that it was important to understand molecular mechanisms involving stress perception, signal transduction, and transcriptional stress tolerance regulation, which could help engineer tolerance to multiple stresses in crop plants. They also mentioned that advances in the molecular biology of stress responses in tolerant organisms introduce the potentials of stress tolerance genes in agricultural programmes, not only to ensure survival but also to ensure productivity.

Despite advances in understanding tolerance and achievement mechanisms in plants, there are still important issues to consider and study in the future, such as the effect of the expression in other crops, the impact on ecosystem productivity, and so on for a realistic agronomic assessment. However, a comprehensive and accurate view held about the problem and methods that are currently available for deepening the study as a complex phenomenon have changed the outlook and have opened up many more possibilities for improving crop tolerance to salinity.

In general there is much work to do to improve crops because the problem is increasing with salinity, drought, and other worldwide factors that affect crops. We are considering expanding our research on *Paulownia* since it is an important woody

species in reforestation programmes, which can diminish desertification and will be an intercropping species.

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